THE HISTOLOGICAL STRUCTURE OF THE ENTERON OF
NECTURUS MACULATUS.

BY BENJAMIN F. KINGSBURY.

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Awarded First Prize in Animal Histology by the Society.

Benjamin F. Kingsbury, A.B. (Buchtel), M.S. (Cornell), Defiance, Ohio.

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INTRODUCTION.

This investigation has for its object an addition to the knowledge of the digestive tract of the lower *Amphibia*. Naturalists have bestowed upon the various orders of *Amphibia* very different degrees of attention. The structure of the enteron of the *Anura*, or tailless *Amphibia*, has been repeatedly and thoroughly studied. The frog especially, which in other departments of physiology has contributed so much toward the solution of difficult problems, has also furnished one of the chief sources of knowledge of the digestive processes. The investigations of Swiecicki, Partsch, Klein, Langley, Sewall, and others upon the stomach of the frog have contributed much to a knowledge of its glands and their functions while the intestine has figured in almost all of the many investigations which have been made on that portion of the digestive tract during the last fifty years. With the *Urodela*,† or tailed *Amphibia*, it is far different. Of the three orders comprising this group, only two families, represented by three genera, have received more than very general treatment. These forms are *Proteus anguineus* and the salamanders and tritons, all of Europe.

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*This paper, with minor alterations, was presented to the Faculty of Cornell University for the degree of M.S. in June, 1894. A sincere appreciation is felt for the abundant material and facilities placed at my disposal by the University, and for the suggestions and advice of Prof. Gage throughout this investigation.

†The words *Urodela* and Urodele are used in this article with the meaning given them by Huxley (32) and are synonymous with tailed *Amphibia*. Salamander is applied only to members of the family of *Salamandridae* of Europe.

Proteus anguineus, because of the place that it holds as one of the lowest *Amphibia*, and the hesitation that was at one time felt in including it among the *Amphibia* at all, has received considerable attention, and its enteron has been quite thoroughly studied by a series of investigators from Valentine in 1837, to Oppel in 1890. The salamanders and tritons have also been studied more or less thoroughly, generally in connection with the frog and toad, by Klein, Partsch, Haidenhein, Langley and others, who have made histological studies upon the digestive tract, so that the structure of the enteron of the forms of this family of urodeles is quite well known.

The remaining families and their genera, including many forms confined solely to America, and of peculiar interest, many of them, because of their low zoological rank, still await the investigator, and promise a rich field for study.

Of these typical American forms, among others, *Necturus* is particularly interesting. The fact that it is one of the lowest of *Amphibia* and the only near relative of the well known *Proteus* of Europe, together with its localized habitat in North America alone, should make and has made it in many of its anatomical features, a much studied form among *Amphibia*, and one especially proper for the investigations of American zoologists.

While much of its anatomy has been more or less studied by naturalists, chiefly European, its enteron has received but little attention. In the long list of those who have published accounts of investigations upon the anatomy of *Amphibia*, only three names appear of persons who have treated of the histology of the enteron of this form.

(1) Van der Hoeven, 1857, (71) who has worked upon *Necturus* to some extent, chiefly upon its musculature, spoke of the epithelium of the mouth, and mentioned and described the taste-bulbs.

(2) Hoffmann, 1878, (30) in Bronn's Thierreich, in the chapter upon the digestive tract of *Amphibia*, referred to *Necturus* several times, mentioning its taste-bulbs and its large esophageal glands. As he cites no authority, the statements must be regarded as based upon investigations of his own.

(3) Gage, 1885, (20) in a paper before the American Society of Microscopists, speaks of the stratified condition of the oral epithelium, and the total absence of cilia upon it.

In these three is comprised all published account known to me of investigations upon the enteron of *Necturus*, and to in some measure supplement this very inadequate knowledge of the digestive tract of this interesting form, these investigations were undertaken. They are almost purely histological, the gross anatomy being discussed only in so far as it is necessary for the histology. The entire enteron from mouth to anus is studied with the following limitations :

The intermaxillary gland which opens into the oral cavity is not discussed. Wiedersheim (73) has made quite an exhaustive study of this gland in a number of *Amphibia*, of which *Necturus* is one. (2) No account is taken of the teeth. (3) No attempt has been made to study the structure of the liver or pancreas, except in the number, arrangement and structure of the bile and pancreatic ducts. (4) The cloaca is merely considered in so far as it is the outlet of the digestive tract.

The state of digestion which so materially affects the epithelium and glands of the digestive portion of the enteron has been carefully considered in each specimen, and the corresponding differences in minute structure noted.

The physiology which is intimately connected with the histology has been studied in so far as it is necessary to understand the structure, but in no case with the thoroughness which it deserves.

The alimentary canal of *Necturus* is quite simple. However, there are five clearly marked tracts, recognizably distinct in structure, namely : (1) the mouth cavity, (2) the esophagus, (3) the stomach, (4) the intestine, (5) the cloaca.

THE MOUTH CAVITY.

The mouth cavity comprises that portion of the enteron extending from the mouth-opening to the esophagus. The mouth is quite large and when opened exposes the entire oral cavity. The roof of the mouth or palate is nearly flat, without depression or elevation save the rows of teeth. On the floor of the mouth behind the teeth is found the short, fleshy, and apparently functionless tongue, closely attached to the floor of the mouth and only free at its tip. On each side are three recesses, between the gill arches, the caudal two, leading to the gill slits, while the first is blind and has no gill opening.

The lining epithelium of the mouth is stratified, (Figs. 1–4) as it is in all *Amphibia* and higher forms, and is non-ciliated as was shown by Gage (20), thus forming an exception to *Amphibia* in general. This is the condition in *Proteus* (Oppel, 53), *Siredon* (Carriere, 7), and *Cryptobranchus* (*Menopoma*,) (Gage, 20), upon whose oral epithelium no cilia could be detected, and is probably true of all aquatic Amphibia (Gage. 20). The epithelium upon the palate, floor of the mouth in front of the tongue, and tongue is of about the same thickness, and is formed of 4–5 layers of cells. In the depressions between the gill arches, and on the under side of the tongue, the epithelium is much thinner and there consisted of but two or three layers.

As in other *Amphibia*, two kinds of cells are found composing the epithelium, goblet and ordinary epithelium cells. The ordinary epithelial cells are of an irregular polygonal outline, with a more or less spherical nucleus. The cells of the ental layer are somewhat elongated in form as is also the nucleus. The cells of the surface layer (Fig. 4) are characterized by the so-called cuticular border of the epidermic cells of fishes and some *Amphibia,– Proteus* (Oppel, 53), *Siredon* (Carriere, 7). This border has been observed in the surface cells of the mouth of *Proteus* (Oppel, 53), *Siredon* (Carriere, 7), and upon the unciliated surface cells in the mouth of the salamanders, frogs, and toads (Hoffman, 30,) and is presumably more or less universal among *Amphibia*. It presents the appearance of vertical striae in the fresh condition, and is readily differentiated by the use of stains.

Goblet cells (Figs. 2 and 8) are found in all parts of the epithelium, and are perhaps most abundant on the floor of the mouth in front of the tongue. They are situated in every layer of the epithelium, and vary exceedingly in form from great bubble-like cells with flattened nuclei (Fig. 8), to slender, elongated forms which extend throughout the entire thickness of the epithelium (Figs. 1 and 2). The nucleus of the cell is situated in the foot and appears more or less spherical, and is stained brown with Ehrlich-Biondi stain, while the other nuclei are stained blue or green. The theca of the cell is as variable in its size, as are the cells themselves. The reticulated structure, both of the foot and the theca of the goblet cells, as described by List (42) was very evident in isolated cells and sections stained with the Ehrlich-Biondi

stain. No areal distribution and limitation of the various forms of goblet cells was observed, except that upon the dorsum of the tongue they appeared not to extend deeper than the second or third layer.

Beneath the epithelium of the mouth cavity were found papillae of connective tissue bearing on their summits bulb-like organs much resembling the sensory organs in the mouth of fishes and higher forms (Fig. 6). These were found in all parts of the palate, under, and upon the dorsum of the tongue where they were the most abundant. The presence of these sensory organs in the mouth of Necturus was noted by Van der Hoeven (71), and Hoffmann (30), by the latter of whom they are compared to the sense organs of the lateral line of fishes and Amphibia. Bugnion (6) found organs of apparently the same structure in the oral epithelium of two other low *Amphibia, Proteus* and *Siredon*. In the *Anura* and *Salamandridae* (Hoffman, 30), no such are found. However, with the larval frog (Schulze 61) and the larval triton (Malbranc, 45) bulbs apparently identical in structure are present, —a very interesting fact considered in connection with the other developmental changes in the higher *Amphibia*. The subepithelial papillae of connective tissue are very marked and extend through about one-third of the thickness of the epithelium, without, however, any corresponding elevation of the surface cells, which in some cases nearly cover the bulb, leaving but a small opening at the top (Fig. 6). The bulb is more or less roughly oval in shape, and is composed of long slender cells with oval nuclei situated in the middle or the base of the cells (Fig. 7). Compared with the sense organs of the lateral line found upon the head, they apparently differ from them only in size, being much smaller, and are situated upon papillae, while the sense organs of the skin are sessile.

Numerous vacuoles (Figs. 4 and 5) were observed in the epithelium, containing highly stained elements some of which took a nuclear stain, others a protoplasmic stain, and often an evident leucocyte or several leucocytes. As similar vacuoles were found in almost all portions of the enteric epithelium, a consideration of them will be postponed until these portions have been discussed. Single leucocytes were however seen in all portions and depths of the oral epithelium, and in the sub-epithelial tissues, where they

occurred sometimes in great numbers somewhat suggesting lymphoid tissue. They could easily be detected in Ehrlich-Biondi stained sections by the bright green of the nucleus and the dense red granular protoplasm, thus probably identifying them with the eosinophile leucocytes of Ehrlich. Both mono- and multinucleated forms were observed, and leucocytes with much or little protoplasm.

The epithelium of the mouth resembles greatly the epidermis of the outer skin, differing from it in several particulars, *i. e.*, in the absence of the subepithelial mucous glands, the large cells of Leydig (39) and pigment cells in the epithelium, and in the presence of goblet cells. Furthermore, the epidermis is somewhat thicker and consists of 7–8 layers of cells. The transition from the outer skin is gradual ; the glands of the cutis and the cells of Leydig cease upon the outer side of the lip, while the first goblet cells appear within the line of the teeth.

ESOPHAGUS.

The esophagus may be said to begin immediately behind the second gill slit, and is continued caudad and slightly to the left as a short broad tube, but slightly more contracted than the mouth cavity. The ental surface is thrown into persistent longitudinal folds (Fig. 9), and is clothed with a simple columnar epithelium, which is ciliated throughout the length of the esophagus. The form and size of the epithelial cells vary much with their position upon the mucosa. Upon the lateral surfaces of the folds, they are set obliquely, and often present a curvature of nearly 45 degrees. The size of the cells also varies with their position upon the folds, those clothing the depressions are usually about one-half the length of those upon the crests of the folds.

Three kinds of cells compose the epithelium, ciliated columnar cells, goblet cells, and spindle-shaped cells (Fig. 15). Ciliated cells are the most abundant, and give the epithelium its character. The nucleus which is oval is usually situated near the free end of the cell. The goblet cells were found plentifully among the ciliated cells. Their number appears to vary considerably in different individuals. The theca is a well defined cup situated upon the slender foot, and from it the finely granular mucous

mass was often seen protruding. Between these two kinds of cells are found smaller spindle cells which do not project to the free surface. The nucleus is oval and situated close to the base of the cell. These undoubtedly represent the so-called substitution cells (Ersatzzellen). Their position in the ental portion of the epithelium, and the fact that the nuclei of the goblet cells are generally more deeply situated than those of the ciliated cells, give to the epithelium a stratified appearance.

In the portion of the esophagus cephalad of the stomach are found a number of large saccular glands (Fig. 11). They measure about ⅔ of a millimeter in diameter, and in the fresh specimen may easily be detected by the unaided eye as clear spots in the wall of the esophagus. Although I have not counted them in any specimen, I should estimate their number as between twenty and thirty. In structure they are simple sacks lined with a single layer of cells, of which two kinds may readily be distinguished, mucous cells, and the secreting cells of the gland. The mucous cells (Figs. 11 and 12) occupy the region surrounding the neck of the gland. They are large columnar cells with the nucleus situated in the basal end. Within the cell a very fine network is discernible. The other cells (Figs. 11, 13 and 14) vary much both in different glands and in the same gland, from a cubical or even flattened form to a columnar one. They stain readily with eosin and fuchsin, thus differentiating them from the mucous cells which stain but lightly.

Among the other *Amphibia*, esophageal glands have been observed according to Hoffmann (30) only in frogs and toads and *Proteus*, while they are entirely wanting in *Siredon pisciformis*, the *Salamandridæ*, and in *Bombinator igneus* and *Cystignathus ocellatus* among the *Anura*. Upon other *Amphibia* it would appear no investigations have been made. I myself have sectioned the esophagus of *Diemyctylus viridescens* and found glands wanting. In *Proteus* and *Necturus* these glands are evidently very similar and are simple saccular glands. In the frogs and toads the esophageal glands are compound and saccular. In these forms they have been determined as pepsin-secreting glands whose cells contain zymogen granules which are stained brown with osmic acid (Langley, 37). I was unable to demonstrate such granular contents in *Necturus*. I repeatedly treated these glands with

osmic acid both when the enteron was entirely empty and in a
state of fasting, and when it contained food, to detect if possible
secreting granules in the cells, but found no trace of them. I
was particularly anxious to determine their presence or absence
in *Necturus* since Oppel (53) finds in the very similar esophageal
glands of the *Proteus*, granules in the cells which he compares to
the esophageal gland granules of the frog. They were, however,
according to him, much smaller and not nearly as abundant as the
granules in the esophageal glands of the frog. In *Necturus* the
outer portion of the cells was indeed finely granular, but in no
way appeared different in structure from the granular contents of
the goblet cells of the surface epithelium. In some of my prepa-
rations, and particularly in those hardened in Erlicki's fluid, the
so-called mucous globules were seen, both free and in all stages
of exudation from the mucigenous ends of the stomach epithe-
lium (Fig. 18), and in every case similar globules were ob-
served free and in process of secretion from the cells of the
esophageal glands (Fig. 14), which would seem to indicate that
whether pepsin producing or not, mucous was secreted by the
cubical cells of these glands.

It does not appear possible in the present state of knowledge to
declare with certainty the homology of these glands in *Necturus*
with those of the frog. On comparing the orders of *Amphibia*,
esophageal glands are found present in frogs and toads among
tailless *Amphibia*; among tailed *Amphibia*, present in the lowest
and wanting in the highest forms. It is evident from the palæon-
tologic remains and the present wide gulf between the tailless and
tailed *Amphibia* that the latter must have become separated at an
early period of geologic time, and most closely related to them
among the urodeles would be the low *Proteida*. Hence it would
not be surprising to find compound and highly functional glands
of *Anura* present in these as simple glands with their ferment-
producing action weak (*Proteus*) or apparently wanting (*Nec-
turus*). However, the matter is complicated by the absence of
these glands in *Crstignathus* and *Bombinator*, the latter of which
seems in many details of structure to approach the *Urodela*; and
the *Discoglossidæ*, the family to which it belongs, is (Cope, 9)
"the nearest of all the *Salientia* (*Anura*) to the original and now
extinct type which formerly connected that order with the Sala-
manders (urodeles.)"

Of the other urodeles, only a few higher forms have been studied ; others should be investigated, especially the genera *Imphiuma* and *Cryptobranchus*, which appear to be low forms more closely related to the *Proteida* than those already studied. In fact, the whole matter requires the study of far more forms both among the tailless and tailed *Amphibia*, before the homologies of these glands can be determined.

Beneath the epithelium occurred a layer of connective tissue rather denser in the part immediately entad of the epithelium, which doubtless represents the mucosa, the rest representing the submucosa as developed in higher forms. A muscularis mucosae occurs in the esophagus as scattered bundles of unstriated tissue, which become larger and closer together nearer the stomach. The two customary coats of the muscularis externa are found in the esophagus, the longitudinal and circular coats, consisting of plane muscle cells. The longitudinal layer is much the weaker and in the cephalic portion of the esophagus does not form a continuous sheet of tissue but consists of broken bundles, which are united by connective tissue.

STOMACH.

The transition from esophagus, externally almost imperceptible, is upon the ental surface of the wall, very evident by the abrupt termination of the longitudinal folds of the esophagus. The stomach when distended presents no significant folds or wrinkles in its cephalic portion. In the pyloric end, longitudinal folds of the mucosa make their appearance. In the minute structure, however, the transition is found to be more gradual. There is no line of demarcation separating the esophageal epithelium from that of the stomach. The ciliated cells of the esophagus extend caudad here and there among the stomach cells, and cells of the stomach epithelium occur in the midst of the ciliated cells at the stomach end of the esophagus.

The surface epithelium of the stomach consists of simple columnar cells of the type characteristic of stomach epithelium, in *Amphibia* and higher forms, and which are, agreeing with the other structural elements of *Necturus*, large, measuring about 80 mikrons, on the average, in length. The nucleus is oval, and contains 6–8 nucleoli. It is situated at or near the middle of the

cell, so that there is not the appearance of two layers which is presented in the esophageal epithelium. The outer mucigenous border (Fig. 17, a) was very evident in all my preparations, and was clearly demarcated from the rest of the cell by the use of carmine and fuchsin stains. In the fresh state the appearance of fine longitudinal striæ, characteristic of the mucigenous border, was clearly seen.

Nothing is offered toward a solution of the once vexed question of the existence of a membrane upon the outer end of the stomach epithelium cells. The mucigenous border was generally more or less rounded, and often presented a protruding mass which appeared in some cases to be demarcated from the mucigenous border proper. Also in some preparations, as before mentioned, globules of mucous were seen free upon the mucosa and in process of exudation from the ends of the surface cells. (Fig. 18, a). This however could not be regarded as an indication of the absence of a membrane, as it might be ruptured in secretion, as it seems to be in ciliated secreting cells (Van Gehuchten. 70). In no case did I observe a goblet cell in the stomach epithelium, or anything corresponding to an empty theca.

In the surface cells of the stomach epithelium, between the mucigenous border and the nucleus, were observed in greater or less abundance, granules with the appearance and actions of fat (Fig. 23). They stained black or brown-black with osmic acid, and were dissolved in xylol, thus indicating that they were fat granules. Their presence and abundance in the specimens examined showed a peculiar dependence upon the digestive condition of the stomach, though it appeared in some cases to be quite variable.*

*The stomachs of twelve Necturi were examined with the following results:

3. Whose enterons were entirely empty, showed hardly a trace of granules in the stomach epithelium.

1. With a little matter in the intestines just above the cloaca, hardly a trace of granules.

1. Lower half of the intestine full of matter, stomach empty, hardly a trace of granules.

1. Lower half of intestine full of matter, stomach empty, granules very abundant in the stomach.

6. In which the stomach contained partly digested food. (Fish, crawfish) the granules were abundant in the epithelium.

The occurrence of fat in the surface epithelium of the stomach in other animals, has been observed only in special cases. Both Watney (72), and Ogneff (52), mention the presence of fat in the stomach epithelium of young Mammals which are still suckling. Oppel (53), speaks of the presence of granules in the surface epithelium of the stomach of *Proteus* which "brown" in osmic acid ; but he offers no suggestion as to their nature, or the cause of their presence. They were found only in the stomach. In the cells of the stomach glands of the frog, fat granules were observed at times by Langley (37). No explanation of their presence could be given.

The glands of the stomach of *Necturus* are of the two kinds generally present, the so-called cardiac, or oxyntic glands of Langley, and the pyloric glands.

The cardiac glands (Fig. 20) are far the more numerous and occupy the cephalic three fourths of the stomach. In the anterior portion they appear to be mostly bi-tubular and rather short ; further caudad, they become simple tubules, increase in length, and also become more closely aggregated. They are of the structure characteristic of these glands in *Amphibia* in the forms investigated, and consist of a neck, several mucous cells, and the secreting cells of the gland.

The neck varies considerably : It is composed of cubical cells, with more or less tapering free ends. Below the neck come five or six large mucous cells, which are followed by the tubule formed of the secreting cells of the gland. The mucous cells (Fig. 20, d) are very large and easily distinguished. They present the characteristic reticulum, and possess a small nucleus situated in the base of the cell. They are more numerous in the anterior glands, and become fewer caudad. The secreting cells (Fig. 20, e) of the gland constitute the tubule below the bubble-like mucous cells, and appear in every way comparable in structure to the similar cells in the cardiac glands of the frog. They present two zones, an inner granular and an outer clear zone containing the nucleus (Fig. 22). These cells stain readily with eosin, fuchsin, and picric alcohol. In tissue hardened in Erlicki's fluid the granular zone was clearly differentiated from the rest of the cell by the use of the Ehrlich-Biondi stain ; the granules stained very lightly in contrast to the rest of the cell. They will be further

discussed subsequently. By some investigators the secreting cells have been regarded as homologous with the parietal cells of the mammalian stomach glands, while the mucous cells were regarded as representing the chief cells. This comparison appears to be faulty. Recent investigators have shown that in mammals the chief cells contain zymogen granules and are the pepsin producers, while the parietal cells are regarded as supplying the hydrochloric acid of the gastric juice. In *Amphibia* both functions seem to reside in the same cells, which contain the zymogen granules which produce the pepsin, and stain very similarly to the parietal cells. Others have made the mucous cells of the glands comparable to the surface cells of the stomach, from which they differ in shape and appearance, though both are undoubtedly mucous secreting. What the origin of these cells might be, and their relation to the other cells of the gland, remains undetermined.

The region of transition from the esophagus to the stomach is marked by some rather interesting and peculiar glands. No transitional forms between the esophageal and stomach glands occur, at least in the adult form. Oppel (53) speaks of finding in the *Proteus*, glands which he thought might be considered as transitional. I observed no such in *Necturus*. This is different from the condition in the frog, in which the esophageal glands pass gradually into the cardiac glands of the stomach, by a diminution of the number of tubules, and these transitional forms contained in the same gland, the cells of the esophageal glands and those of the stomach glands, side by side. (Langley, 37). There do occur, however, in the gastric end of the esophagus, glands which seem to resemble similarly situated glands of the salamanders which have been regarded as analogous to the esophageal glands of the frog. These glands in *Necturus* (Fig. 21), consist of a large body composed of mucous cells and from which extend two, three, of four tubules of secreting cells. In some glands the mucous cells were even more numerous than in the one figured. They were very large with a small nucleus situated in the basal end. A fine network could be seen in the body of the cell. These glands first occur, as was said, in the esophagus, and even before the esophageal glands have ceased, so that in the same transection, the two may be seen opening upon the ciliated surface. They occur in clusters which are separated from

each other and from the stomach glands by connective tissue. In the salamanders of Europe (including the tritons) quite similar glands are described. Klein (33), spoke of a ring of acinous glands in the esophagus of the *Triton* which he regarded as similar in structure to the esophageal glands of the frog, and Langley (37) mentioned them as differing from the anterior cardiac glands only in the greater numbers of mucous cells and tubules. Whether or not these glands are homologous with the esophageal glands of the frog would be difficult to determine, and in *Necturus* the question of the homology of the esophageal glands is involved. The esophageal glands of the frog are pepsin-forming glands, and the zymogen granules contained differ from the granules in the cardiac glands in being larger, and colored a yellow brown with osmic acid, while the granules in the stomach glands are turned a black brown. Both in the *Triton*, (Langley, 37), and in *Necturus* the granules contained in these several-tubuled glands are identical in appearance with the granules of the anterior cardiac glands, so that in this respect no resemblance appears between these glands and the esophageal glands of the frog. However, while pepsin is produced most abundantly in the esophageal glands of the frog, it is also secreted from the stomach glands, though this is disputed by some ; and in forms in which, esophageal glands are entirely wanting, must be formed in the stomach exclusively ; so that the difference between the esophageal and stomach granules in the frog would seem but superficial.

As in the *Triton*, so in *Necturus*, a marked difference in size was found between the granules in the extreme cephalic and caudal glands. With osmic acid the granules were stained a light brown in all the cardiac glands.

In this transitional region were also observed five or six sacks formed of large mucous cells alone, resembling the bodies of the glands of this region, but with no tubules attached. They often appeared almost as mere depressions of the mucosa. These might perhaps be regarded as indicating the origin of the glands.

The pyloric glands (Fig. 25), occupy a much more limited area, and are found only in the caudal fourth of the stomach. In a fasting stomach the region occupied by these glands is very clearly demarcated from the rest of the stomach by the different appearance of the mucosa, it being quite transparent, while the rest of

the stomach in the fresh condition is yellow and opaque. There is no gradual transition from the cardiac glands to the pyloric glands by a diminution of secreting cells such as occurs in the stomach of the frog (Langley, 34). The long tubules of the cardiac glands stop quite suddenly, and the short pyloric glands succeed. These glands have been compared to the necks of the cardiac glands, and their cells stain quite similarly. The cells are quite transparent; the nucleus is large and is surrounded by but little protoplasm, which extends between the adjacent cells as long processes. They contain no granules (Fig. 25). The large mucous cells which are occasionally found in the bottom of the pyloric glands of the frog (Langley, 34), thus strengthening the theory of their homology with the necks of the cardiac glands, were not found in *Necturus*. The pyloric glands are not so closely packed as are the cardiac glands, and extend to the pylorus, and would seem to be continued as the glands of the intestine.

The nature of the secretion of the glands of the stomach was not investigated; undoubtedly it is similar to the gastric juice of the frog, and consists chiefly of pepsin ferment acting, in an acid fluid. Milk which was injected into the stomach of a *Necturus* was formed into a thick clot, indicating the presence of the curdling ferment, or rennin of mammals. According to Foster (Text book of Physiology), this ferment is present in the stomach of most animals. Whether the zymogen of this ferment is stored in the cells in the form of granules which can be distinguished from those which produce the pepsin ferment would be interesting to determine. Langley (36), found the zymogen of rennin in the frog in both the esophageal and gastric glands.

Numerous gigantic vacuoles containing leucocytes were found in the epithelium of the stomach and in the bodies of the pyloric glands. They will be discussed with similar occurrences in the intestinal epithelium.

A muscularis mucosæ was evident in the stomach, and in the cephalic portion was composed of a single coat of longitudinal fibers. In the pyloric portion of the stomach there was added a second inner circular coat. The circular and longitudinal coats of the muscularis externa were very well developed and increased in thickness, especially the circular coat, as the pylorus was approached. The plain muscle cells of which they are composed

had the characteristic appearance of all involuntary muscle, agreeing with the other structural elements of *Necturus* in being quite large. In length they possessed an average measurement of about 1.8 m.m., with 65 mikrons for the corresponding dimension of the nucleus. The plain muscle cells in all portions of the enteron are of approximately the same size, except in the circular coat of the pylorus, where both cell and nucleus are shortened by half of these dimensions. At the cardiac end of the stomach the two coats are closely connected by bundles of fibers which run from the one coat to the other, and which may represent the oblique layer of the stomach of Mammals.

INTESTINE.

The intestine of *Necturus* is quite simple, both in form and structure. When relaxed, it is folded once or twice, being much more convoluted than the intestine of *Proteus*, and not so much so as in the higher *Amphibia*. No demarcation of a rectum such as occurs in the higher *Amphibia* is observable in *Necturus*. Feces tend to accumulate in the portion cephalad of the cloaca, giving the appearance of a normal enlargement, but when entirely empty, there is no sudden or gradual increase in size in the intestine which would indicate the existence of a separate tract. In the minute structure the change is gradual throughout the intestine from stomach to cloaca. If by analogy with the higher forms of *Amphibia* in which a rectum exists, the cloacal end of the intestine should be called a rectum, as it is by Hoffman (30), the determination of the point at which the small intestine ends and the rectum begins would be difficult. It would rather appear that the intestine had not yet become differentiated into two distinct tracts.

The ental surface of the intestinal wall is marked by a number of longitudinal folds of the mucosa, which run in a zigzag manner. They are persistent in the distended intestine and are very large when it is contracted. (Fig. 30.) They doubtless correspond functionally to the villi of mammals and in them the absorption of fat appears chiefly to take place. By treating with osmic acid an intestine in which fat absorption was taking place, the presence of fat globules in the epithelium and connective

tissue of the folds almost exclusively, was evinced. In structure, however, they are more nearly comparable to the valvulae conniventes of man.

The epithelium (Figs. 32, 33) of the intestine, like that of the esophagus and stomach, consists of a single layer of cells which are of the two kinds characteristic of intestinal epithelium namely, the columnar cells and goblet cells. The first are the more abundant in the epithelium and vary considerably in size and shape according to position. Upon the sides of the folds the cells are, as in the esophagus, bent to a considerable angle and are set upon the mucosa obliquely. They are long and slender upon the crests of the folds, and broad and short in the depressions between the folds, and vary also in the basal end of the cell from very slender and tapering to the typical columnar cell of equal thickness throughout its length. The nuclei of the columnar cells are situated generally near the middle of the cell.

The striated border is quite broad and its vertical striae evident. It is clear and refractive in appearance and stains but lightly. In the cephalic portion of the intestine it is quite broad, and is much thinner in the caudal end. If, indeed, this border has an intimate connection with the process of fat absorption as held by some, its much stronger development in the cephalic portion of the intestine where the absorption of fat is most active would seem consistent.

Goblet cells (Fig. 34) occur in the epithelium throughout the length of the intestine, but are less abundant in the cephalic portion. The theca is rather small and bulb-like, with a constricted top. The nucleus is situated generally in the base of the cell, and the characteristic reticulum of the cell-body is easily seen. The number of goblet cells seems to be somewhat variable, and also varies with the region of the intestine. In the cephalic portion they are less abundant; proceeding caudad, however, the theca becomes larger, in proportion to the size of the cell, and the cells themselves more numerous until at length, just cephalad of the cloaca, the epithelium is largely composed of goblet cells.

The transition from the stomach to the intestine is as in Mammalia (Watney 72), marked and sudden. The very well developed muscular coats of the pylorus are thinned abruptly to form the corresponding coats of the intestine. The epithelium of the

stomach stops quite suddenly, and the striated cells of the intestine with goblet cells interspersed, succeed.

The glands of the intestine which occur in other *Amphibia*, were also found in *Necturus* (Fig. 27). They occur throughout the intestine and are situated, as in other *Amphibia*, almost entirely in the depressions between the folds of the mucosa (Figs. 30 and 31), though occasionally found upon the lateral surface of the folds. In form and appearance they resemble the glands of the pylorus. A resemblance is also seen in the cells composing them. They are cubical in shape and transparent. The nucleus is spherical, with but little protoplasm surrounding it, which is often prolonged into processes extending between adjoining cells.

Recently in the investigations of Bizzozero (2) and Nicolas (47) upon the *Triton* and salamanders, doubt has been thrown upon the character of these groups of cells as glands. By these two they are regarded as nidi of young cells, from which new epithelium cells are produced. My study of them in *Necturus* would however lead me to regard them as glands. The arrangement of cells as if surrounding a lumen, and indeed a lumen itself, which Nicolas declared did not exist in these structures, could be seen upon almost all of my sections in some of the glands. I was unable, however, quite satisfactorily to demonstrate the existence of a neck, although it seemed in numerous glands to be quite well indicated. Hoffmann (30), however, speaks of their almost circular opening upon the surface epithelium of the intestine. The glands are not all of the size and shape of the one figured. Many consisted of but few cells, with no definite arrangement. Since neither Bizzozero nor Nicolas mention or figure any at all, comparable in glandlike appearance to the intestinal glands of *Necturus*, such probably do not exist in the *Salamandridae*. Bizzozero regards these groups of cells in the *Triton* as phylogenetically identical with the crypts of higher forms of animals; yet, to find such in *Necturus* more developed than in a higher family of *Amphibia*, would appear strange.

LEUCOCYTES.

In the intestine occurred vacuoles containing leucocytes, and which were similar to those mentioned as occurring in the epithelium of the mouth and stomach, consideration of which, because

of their size and appearance, has been omitted heretofore, that they might receive a separate treatment. The leucocytes contained in these vacuoles in the epithelium in all parts of the enteron, so monstrous as they are, might almost be regarded as a distinct class. Their nucleus is of irregular shape, and is surrounded by a greater or less amount of finely granular protoplasm, which stains but lightly, (Fig. 35 a–e) and thus by the use of a stain which colors the surrounding cells intensely, such as fuchsin, they are easily differentiated. The vacuoles themselves are thus made more apparent, especially as the leucocytes often fail to fill them entirely. That they are subspherical vacuoles and not transected tubes of the nature of lymph spaces perhaps, was determined by means of serial sections. Their size and shape would seem to indicate that they are more or less permanent in the epithelium, and that the leucocytes contained are not merely wandering to the surface, but have a permanent abode upon the digestive highway. The presence of small leucocytes and protoplasmic elements in the vacuoles has been noted before. A study of these is very suggestive and would seem to indicate upon what food the large leucocytes have fed that they have grown so great. The number of small leucocytes and deeply staining masses within these vacuoles is sometimes so great as to apparently fill the vacuole and obscure the presence of the large leucocyte, which yet I think is present, as in less crowded vacuoles it can always be clearly seen; and it is in such vacuoles in which but one or two of these small leucocytes occur, that they can best be studied, and a suggestion obtained of the cause of their presence, namely, that they are engulfed by the large leucocyte with the faintly staining protoplasm. For the study of the vacuoles the Ehrlich Biondi stain was found particularly useful because of the different shades of color obtained by its use. The nuclei of the leucocytes are stained green, the finely granular protoplasm of the large leucocytes, a light pink, and the globules or granules contained in the large leucocyte, a bright red or orange. These globules were also seen in the small leucocytes (Fig. 35, d) and suggest the idea that the independent globules came from the leucocytes, and that these are being disintegrated. Further, the apparent inclusion of both globules and leucocytes within the protoplasm of the large leucocyte would seem to indi-

cate that the small leucocyte has been devoured, and that the large leucocyte is what Ruffer (58) has termed a macrophage. Another circumstance spoken of further on points to the same conclusion.

In the stomach epithelium, as before mentioned, these leucocytes were very abundant and large (Fig. 26). Here the small leucocytes and the globules within them were less often observed, and it is here that the influence of the presence or absence of food upon their size and numbers, and upon the presence of small leucocytes within them, might be most conveniently studied. Whether or not fat absorption takes place in the stomach, as the presence of fat granules in the surface epithelium might seem to indicate, and these leucocytes appropriate to themselves a large portion of it, as Heidenhain states that they do in the intestine, I have not attempted to discover, though several things would make it seem probable. Their protoplasm is given a slaty black color by osmic acid. This was observed in stomachs which had not had food for at least four days, and is not necessarily an indication of the presence of fat. These leucocytes were also found in stomachs which were in digestion, so that their presence, at least, is independent of the digestive state of the stomach.

Similar giant leucocytes have been observed in other animals by numerous writers, and various interpretations of their nature and the cause of their presence in the epithelium offered, but nothing definite is known. In *Necturus* all sizes of these were found, from the small leucocyte with but little protoplasm to the largest measuring about 50 mikrons ; and this would seem to indicate that they are but leucocytes that have wandered into the epithelium, and perhaps dwelt on the fat of the land, literally. But whether they are permanent in the epithelium, and whether they are useful or harmful to their possessor, are matters upon which nothing is known. List (43) mentions the occurrence of vacuoles in the epithelium of the lip of *Cobitis fossilis*, which often contained several leucocytes. Heidenhain (27) finds and figures very similar leucocytes in the intestine of the frog, guinea-pig, and rabbit, which he calls phagocytes, and which devour other leucocytes, and during digestion are found full of fat globules. Bizzozero (2) speaks of the presence of vacuoles in the intestine, which he regarded as due to the disintegration of leucocytes in

the epithelium. These in *Necturus* also doubtless correspond to the macrophages of Ruffer (58) which he found in the intestine, and which devoured other small leucocytes.

Although their presence is frequently noted in the intestine of different animals, nowhere do I find mention of them in the stomach where, in *Necturus*, they are most abundantly and constantly found. Oppel (53), though he speaks of their presence in the intestine of *Proteus*, makes no mention of their occurrence elsewhere in the enteron. In the intestine of *Necturus* they were found throughout its length and were smaller than those found in the stomach. Leucocytes and globules were found in them constantly. In some portions of the intestine they also contained yellow granules. (Fig. 35, e.)

Closely related to the occurrence of the large leucocytes in the intestine, was that of small leucocytes. (Fig. 35, f p.) These occurred throughout the length of the enteron, in the sub-epithelial tissue, sometimes in great numbers and without any apparent cause, and in the epithelium. They were generally intensely stained by the use of the Ehrlich-Biondi stain. These undoubtedly belonged to the class of eosinophile leucocytes of Ehrlich. Other leucocytes occurred whose protoplasm stained but lightly, and whose nuclei were colored an even green. In the intestine were further found, especially in the caudal portion, though they occurred throughout its entire length and were found just below the stomach and at its opening into the cloaca, leucocytes filled with yellow granules. (Figs. 32, and 35 k and l.) These occurred in the epithelium and in the connective tissue, chiefly in the summits of the folds. The nature of the granules was not determined. They were insoluble in xylol, chloroform, or alcohol, and were unaffected by any stain tried. Oppel (53) regarded them in *Proteus* as pigment granules taken up by the leucocytes which then wandered to the surface with their load of waste matter. That indeed they were proceeding toward the surface and not coming from the surface was evident from the position into which the nuclei of the surface epithelial cells were pushed. (Fig. 32, b.) The cause of the occurrence of leucocytes in the connective tissue and epithelium of the enteron is as yet obscure. The theory that they are instrumental in the absorption of fat is not as generally held as at one time. In fact their appearance in

the intestine seemed independent of the presence of food, and they were most abundantly found in an intestine which contained only a small amount of liquid. The investigations of Claypole (8) have shown that the leucocytes absorbed carbon injected into the system and then wandered to free surfaces with their load, the mucous surface of the enteron included. This would undoubtedly explain the presence of many of the leucocytes in the epithelium, especially the yellow-granuled ones.

Besides leucocytes, there occurred in the epithelium of the intestine, globules of matter clustered together (Figs. 33 and 35. r) which stained intensely with the Ehrlich-Biondi stain, some taking a red stain while others took the nuclear green stain. They resembled very much the globules contained in the large leucocytes. Heidenhain (27) regarded such as the remains of disintegrated leucocytes,—a very plausible explanation, especially as in *Necturus* they were observed conjointly with leucocytes in the epithelium, some of which seemed to show the first phases of such disintegration. (Figs. 33 and 35. m·r). If such is the explanation of their origin in the epithelium, those included in the large leucocytes undoubtedly had a similar source, and support is given to the theory that the small leucocytes are being disintegrated within the large ones.

BILE AND PANCREATIC DUCTS.

The bile and pancreatic ducts open into the duodenal portion of the intestine. The liver of *Necturus* is quite large and extends almost throughout the length of the abdominal cavity. In shape it is somewhat flattened dorso-ventrally, and the lateral edges are cut up into a number of small, shallow divisions. The gall bladder is situated upon the right dorsal side, included between two such divisions.

The gall ducts are short so that the liver is quite closely attached to the intestine. The pancreas is well developed, and is rudely star-shaped. From a center near the gall-bladder, five more or less evident divisions extend. (1) A slender tongue of gland follows the splenic vein, while (2) another extends along the mesenteric vein ; (3) a thin layer extends cephalad for a short distance upon the dorsal surface of the liver, while (4) a fourth

division accompanies the bile ducts to the intestine. (5) The largest lobe, however, runs cephalad along the intestine almost to the pylorus. The pancreatic ducts open into the intestine at two places, by a single large duct just below the pylorus, and secondly, by two smaller ducts which join the bile duct, and open further caudad. There are four bile ducts in *Necturus* which anastomose with the cystic duct and the two posterior pancreatic ducts and open upon the mucous surface of the intestine by three openings. The arrangement of the bile and pancreat ducts, (Fig. 38), resembles very much that of *Proteus*, (Oppel 53), in that the pancreatic ducts open into the intestine in two places, in one of which in conjunction with the bile ducts as in *Necturus*. In *Necturus*, however, the number of ducts which open into the intestine is much smaller. In two of the higher urodeles (Wiedersheim 75), there are two pancreatic ducts one of which united with the two bile ducts to form one duct ; so that *Necturus* in the arrangement of these ducts would hold an intermediate position. The ducts exhibit the characteristic structure of bile and pancreatic ducts, and are lined with columnar cells with the nuclei situated in the basal end of the cell. I was unable to detect cilia upon them.

CLOACA.

The epithelium of the intestine passes gradually into that of the cloaca, by the increase in the number of goblet cells (Fig. 37) of which the cloacal epithelium in the cephalic portion is almost entirely composed. The goblet cells decrease in number further caudad, and at about the middle of the cloaca the epithelium becomes a single layer of cubical cells. A second layer is added farther caudad, and thus the epithelium gradually thickens to merge into the stratified epithelium upon the lip of the cloaca. In the male *Necturus* quite an extensive patch of ciliated cells occurs immediately caudad of the ureters and spermaducts. These ciliated cells occur upon both the ventral and dorsal sides of the cloaca, but extend much farther caudad upon the ventral side, chiefly upon an elevation of the cloacal wall, which further caudad breaks up into a number of thin high ridges. In the cloaca of the female, no cilia were found.

In the presence of cilia *Necturus* differs from *Proteus*, its near relative, in the cloaca of which no cilia have as yet been found (Hoffmann 30). In the higher tailed *Amphibia* ciliated areas occur (Haidenhain, 29).

In my study of the cloaca, there was also discovered, by chance as it were, a fact which although it is not connected with the immediate purpose of this investigation, it might be well nevertheless to mention, namely,—the presence of zoosperms in one of the glands in the cloaca of the female *Necturus*. The glands (Fig. 36) in which those zoosperms occurred were flask-like with a large body and small neck, and were found, forty or more in number, just caudad of the openings of the oviducts in the dorsal wall of the cloaca. They doubtless correspond to the glands of the female *Urodela* described by Leydig (Hoffmann, 30) as cylindrical tubes which gradually become larger toward their end, and to the *receptacula seminis* of Siebold (30) which he found in the cloaca of the Salamandridae. The presence of zoosperms in the cloaca of the female *Necturus* indicates that the eggs are fertilized as laid, as has been shown to be the case in a number of Urodeles. Four adult female *Necturi* were examined and the presence of zoosperms ascertained in each case. The *Necturi* were taken in the winter about the first of January.

SUMMARY.

So far as these investigations might have bearing upon the relative position of *Necturus* among *Amphibia*, comparison is unsatisfactory because of the lack of any investigations upon forms most akin, except indeed *Proteus* of Europe. Compared with this form the evidences of close relationship which an investigation of the structure of the enteron yield are very conclusive. The agreement between the two has been spoken of repeatedly in the foregoing text. *Necturus* appears higher in the scale than *Proteus* (a) in the greater development of its stomach glands. According of Oppel, (50), the secreting cells of the glands are generally 3–6 in number. (b) in the more convoluted state of the intestine ; (c) in its bile and pancreatic ducts.

Between *Necturus* and the *Salamandridæ*, the differences in enteric structure which exist are in the main indicative of the higher

rank of the latter, and appear in the following : (1) The greater external demarcation of the esophagus from the stomach, and the existence of a rectum, together with the more convoluted condition of the small intestine. (2) The presence in the larval and absence in the adult *Salamandridæ* of sensory organs in the mouth resembling those of *Necturus*. (3) The arrangement and number of the bile and pancreatic ducts.

The significance of the absence of esophageal glands in the higher Urodela is obscure. An investigation of the intermediate forms might render it more apparent. In the presence of a ciliated tract in the cloaca, *Necturus* agrees with the higher urodeles.

The general results of the investigation may be summarized as follows :

(1). The mouth, esophagus, stomach and intestine are well defined tracts. The existence of a rectum as a separate tract was not evident.

(2). The oral mucosa is clothed with a stratified epithelium which is non-ciliated, as in the other aquatic *Amphibia* so far investigated.

(3). The esophagus is clothed with a columnar, ciliated epithelium.

(4). Glands occur in the esophagus which are simple alveoli.

(5). Fat granules were found in the surface epithelium of the stomach with an apparent dependence upon the state of digestion.

(6). Cardiac (oxyntic) and pyloric glands occurred in the stomach. Glands of (3–4) tubules were found in the caudal end of the esophagus, resembling similar glands in the *Salamandridæ*.

(7) Large leucocytes which contained ingested cellular masses were found in the epithelium in all portions of the enteron. Small leucocytes were abundantly present in and under the epithelium everywhere.

(8). Glands were found in the intestine throughout its length.

(9). The pancreatic ducts open into the intestine at two places. (a) Immediately caudad of the stomach by a single duct, (b) by two ducts which join the bile ducts.

(10). The bile ducts open into the intestine by three openings. They form an anastomosis with each other and the two posterior pancreatic ducts.

(11). The epithelium of the cloaca in the male was found to contain an area of ciliated cells.

(12). Incidentally, glands were found in the cloaca of the female which contained zoosperms, an indication that the eggs are fertilized as laid.

METHODS.

The structure of the various parts of the enteron was studied both by isolation of the elements, and by means of sections through the regions. At all points of transition, either longitudinal sections were made through the part, serial sections made covering the necessary portion, or, as in most cases, both done. Serial sections were likewise made wherever they were necessary for the determination of any point.

Various methods of hardening the tissue were tried, of which mercuric chlorid, picric alcohol, and Erlicki's fluid gave the best results. Of these mercuric chlorid despite the inconveniences in its use, was most generally employed with excellent results. The fluid used was: a saturated solution of mercuric chlorid in normal salt solution (water, 100 c c, Na Cl. $\frac{6}{10}$ grams). Tissue was hardened in this 1–12 hours, and then thoroughly washed for 12–24 hours in 67 per cent. alcohol to which a little gum camphor was added to hasten the removal of the mercuric chlorid. As long as tincture of iodine is decolorized, when added to the alcohol the washing should be continued. Unless the mercuric chlorid is entirely washed out, crystals will form in the specimen.

For the outlines and relations of cells, Erlicki's fluid gave good results. (Formula: Bichromate of potassium, 2.5 parts, sulphate of copper, 1 part, water, 100 parts). For details of structure, as nucleoli of cells, it was found generally to be inferior. The mucous secretion from epithelium hardened in this fluid (see text) was undoubtedly due to the hardener. Tissue was fixed in this from 1–5 days, and was then washed well in water 1–2 hours, and placed in 67 and 82 per cent. alcohols, a day in each, or indefinitely preserved in 82 per cent.

Picric alcohol gave uniformly good results; very delicate stains might be given to tissue hardened in this. The formula is, 95 per cent. alcohol, 250 cc., water, 250 cc. dry picric acid, crystals 1 gram (Gage, 22). Tissue was hardened in this 1–3 days and then

placed in 67 per cent. alcohol 1 day, 82 per cent. alcohol one day, or until imbedded.

Paraffin and collodion were used for imbedding tissue. In the paraffin method the hardened tissue was dehydrated 1 day in 95 per cent. alcohol, placed in chloroform for one day, infiltrated with chloroform paraffin for 4-5 days in an incubator at 40-50° C., and finally imbedded in small paper boxes in hot pure paraffin. The treatment of the sections when cut was as follows : The slide was coated with a thin layer of albumen fixative (White of egg, 50 cc., glycerin 50cc., Salicylate of soda 1 gram), and the sections firmly fastened by heating and coagulating the albumen. The paraffin was then dissolved out with xylol ; this was removed by 95 per cent. alcohol which was in turn displaced by water. The tissue could then be stained with an aqueous stain. If an alcoholic stain was used, the staining followed the 95 per cent. alcohol.

The castor-thyme method (Fish, 15), was employed in sectioning tissue imbedded in collodion. The hardened tissue was dehydrated 12-24 hours in 95 per cent. alcohol, placed in 2 per cent. collodion 1 day (solution of gun cotton in equal parts of alcohol and sulphuric ether), 3 per cent. collodion 1 day, 6 per cent. collodion 1 day, in which it was imbedded in small paper boxes. The collodion was then hardened in chloroform 1-3 hours, and cleared in the castor-thyme oil mixture (castor oil 1 part, red oil of thyme 3 parts) in which the sections were cut. When transferred to the slide, the superfluous oil was absorbed from the sections and the collodion melted with alcohol and ether (equal parts), fastening the sections to the slide. The oil was then entirely removed from the sections by 95 per cent. alcohol, after which the slide was passed through 70, and 35 per cent. alcohols to water.

Of the stains tried, the following were found most serviceable : hematoxylin, eosin, picric alcohol, hydrochloric acid carmine, and the Ehrlich-Biondi stain.

The last was very generally used and gave excellent results especially in the study of glands and leucocytes, because of its high selective power. The stain as prepared by Dr. Grübler of Leipzig, (Sat. aq. sol. of methyl green, 50 cc., sat. aq. sol. acid fuchsin, 20 cc. sat. aq. sol. of orange, 100 cc.), (Lee, 38), was diluted 50 times with water and allowed to act 2-3 hours. The stain was washed out and the sections dehydrated with 95 per cent. alco-

hol, cleared in xylol or carbol xylol Carbolic acid 1 part, xylol 3 parts : nuclei are stained blue or green, the cell body red, connective tissue purple, and muscular tissue orange. This stain acted best upon tissue which had been hardened in mercuric chlorid or Picric alcohol. The relative intensities of the nuclear and protoplasmic stains may be altered by changing the proportions of the componant stains. Further, if the alcohol with which the stain is washed out be slightly acid, the methyl green stain will become faint in the result, and if alkaline, the fuchsin will be faded.

Hematoxylin used in an aqueous solution, (Gage 23), gave the best results as a nuclear stain. Eosin or picric alcohol was used with it as a counterstain.

Hydrochloric acid carmine was also employed with good results.

In the study of the glands of the esophagus and stomach, and for the detection of fat in the epithelium, osmic acid was used. Tissue was hardened in 1 per cent. osmic acid for 24 hours, washed and placed in a strong solution of gum arabic for an hour or so, and cut by means of a freezing microtome, or free hand. When cut free hand, however, it was generally further hardened in 50 per cent. alcohol 5–12 hours, and 70 per cent. alcohol 1 day.

For the dissociation of the epithelium cells, were employed : (1) Müller's fluid, diluted with an equal volume of water, (2) ¼ and ¹⁄₁₀ per cent. osmic acid, and (3) picric alcohol diluted with an equal volume of water. Picric alcohol was found serviceable in the isolation of ciliated cells.

For the demonstration of the muscular layers of the enteron, nitric acid (20 per cent.) was used. The tissue is allowed to macerate for one day or until the muscular coats can be easily separated from each other. For obtaining dissociation of the individual cells, the maceration should be continued for a day longer, or until the cells separate readily. 35 per cent. caustic potash was also employed in obtaining dissociated muscle cells. After 10 or 15 minutes in the caustic potash, the tissue should be tested every few minutes that the maceration may not proceed too far. When the cells separate from each other readily, the whole may be removed to 60 per cent. acetate of potassium, which prevents further maceration.

Glycerin and glycerin jelley were used for mounting dissociated tissue and sections cut without embedding. Xylol balsam was employed for all other sections.

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THE EXPLANATION OF THE PLATES.

The outlines of all figures except Fig. 38, Plate VIII, were drawn with the Abbé camera lucida, and details were put in freehand ; Fig. 38 is purely diagrammatic. To every figure the scale of measurement is subjoined. The method of preparation of the tissue, the stain used, and the magnification of the drawing are given in each case.

PLATE I.

FIG. A. Young *Necturus*. Dorsal aspect. From the Proceedings for 1882.

FIG. B. Diagrammatic drawing of an adult *Necturus*, 41 c.m. long, from the ventral aspect to show the enteron in situ. The heart and liver have been removed ; the latter is shown in broken outline. Other organs and the mesenteries are disregarded for sake of simplicity. The differences in proportions between the young and old forms, as shown in Figs. A and B, is very striking.

L. = Lungs.	S. = Stomach.
E. = Esophagus.	B. = Branchiae
I. = Intestine.	U. = Bladder.
A. = Vent.	C. = Cloaca.

PLATE I.

A   B
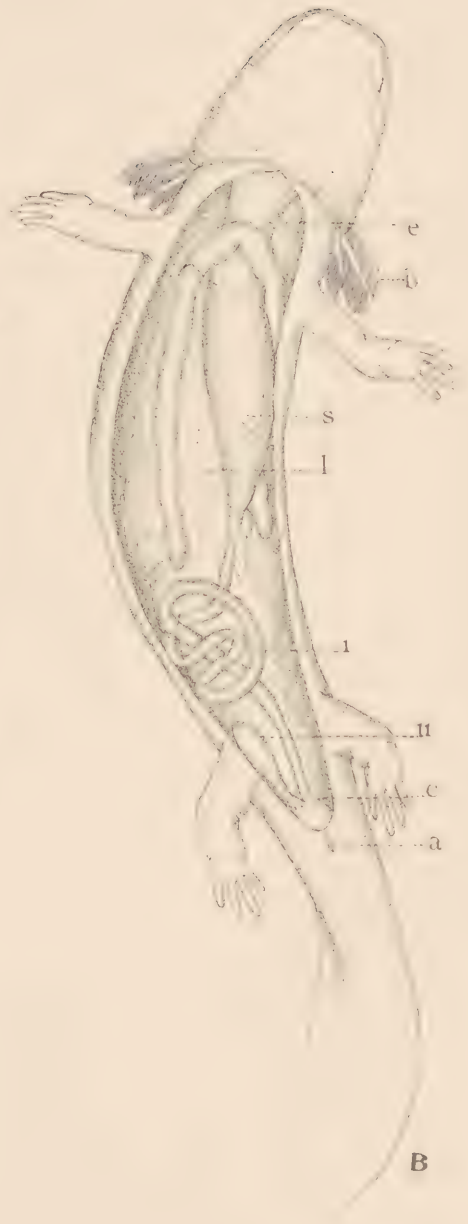
PLATE II.

Figures 1-5 were drawn at a magnification of about 300 diameters, which is indicated by scale A. The magnification of figures 6 and 7 is about 165, according to scale B.

FIG. 1. A vertical section through the epithelium of the roof of the mouth mercuric chlorid hardening. Ehrlich-Biondi stain. (a) Cuticular border. (b) Goblet cells with intracellular network.

FIG. 2. Goblet cell from the oral epithelium. (a) Theca of the cell. (b) Reticulum. (c) Nucleus. Hardened in mercuric chlorid Ehrlich-Biondi stain.

FIG. 3. A vertical section through the epithelium of the dorsum of the tongue. (a) Cuticular border of the surface cells. (b) Vacuole containing leucocytes. (c) Elongated cells of the basal layer. (d) Corium of close connective tissue. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 4. Surface cells from the oral epithelium. (a) Cuticular border. (b) Nucleus of the cell. Isolation in $\frac{1}{10}$ osmic acid.

FIG. 5. A vacuole from the oral epithelium containing leucocytes. (a, a, a) Nuclei of leucocytes. (b, b) Red globules. Hardened in mercuric chlorid Ehrlich-Biondi stain.

FIG. 6. A sensory organ from the dorsum of the tongue. (a) Epithelium (b) Corium of close connective tissue. (c) Sensory organ, situated on a papilla of connective tissue. Attention is called to the arrangement of the cells surrounding the papilla. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 7. Isolated cells from a sensory bulb in the oral mucosa. Isolation in $\frac{1}{10}$ osmic acid.

PLATE II.
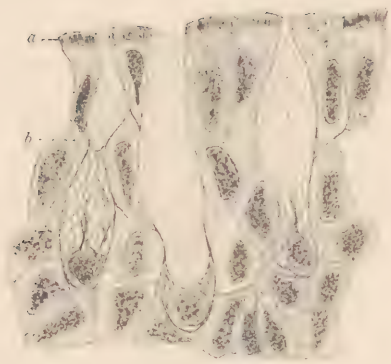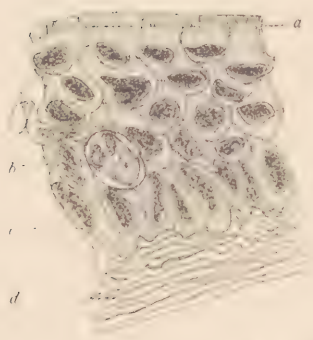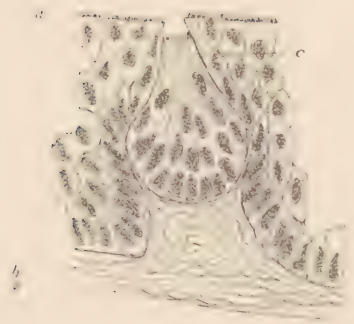
PLATE III.

FIG. 8. A bubble-like goblet cell from the oral epithelium of the floor of the mouth showing the large theca (a) and the small cell-body (b). Isolation in $\frac{1}{10}$ osmic acid. ($\times$ about 300).

FIG. 9. ($\times$ 20). A transverse vertical section through the wall of the esophagus. (a) Ciliated epithelium. (m) Connective tissue of the submucosa. (c) Bundles of plain muscle of the muscularis mucosæ. (d) Circular coat of the muscularis externa. (e) Longitudinal muscular coat. Hardened in Erlicki's fluid. Stained with Hematoxylin and picric alcohol.

FIG. 10. ($\times$ about 165). Epithelium of the esophagus from the crest of a fold. (g) Goblet cells. Hardened in Müller's fluid. Ehrlich-Biondi stain.

Attention is called to the bent form of the epithelial cells.

FIG. 11. ($\times$ 75). Esophageal gland from the caudal portion of the esophagus. (a) Surface epithelium. (b) Mucous cells surrounding the neck of the gland. (c) Cubical cells. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 12. ($\times$ about 450). Mucous cells surrounding the neck of the esophageal gland. (Fig. 11, b) Showing the nucleus situated in the base of the cell and the recticulum of the cell-body, (b). Nucleus of the cell, (a). (c) Subepithelial connective tissue. Hardened in Müller's fluid. Ehrlich-Biondi stain.

FIG. 13. ($\times$ about 450). Cubical cells from the body of the esophageal gland. Hardened by mercuric chlorid. Ehrlich-Biondi stain. Scale same as in Fig. 12.

FIG. 14. ($\times$ about 450). Columnar form of cells from the body of the esophageal gland. Hardened in Erlicki's fluid. Stained with Hematoxylin and eosin. Scale the same as in Fig. 12.

Attention is called to the globules exuding from the free ends of the cells in this specimen (a).

PLATE III.
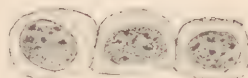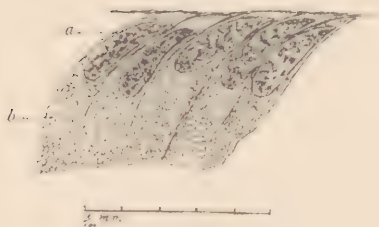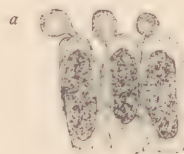
PLATE IV.

FIG. 15. (× about 300). Epithelial cells from the esophagus. 1, 2, Goblet cells; 3, 4, ciliated cells; 5, 6, spindle-shaped cells. (a) Theca of the goblet cell. (b) Nucleus. (c) Cilia. Isolated in equal parts of water and Müller's fluid.

FIG. 16. (× 38). Longitudinal vertical section from the middle portion of the stomach. (a) Mucosa with its surface epithelium and glands. (m) Connective tissue of the mucosa. (b) Muscularis mucosæ. (c) Submucosa (d) Circular and longitudinal muscular coats. (e, e) Blood vessels. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 17. Surface epithelium of the stomach. (a) Mucigenous border showing an ectal and an ental zone. (b) Nucleus. (c) Subepithelial connective tissue. Hardened in mercuric chlorid. Ehrlich Biondi stain. (× about 300).

FIG. 18. Surface cells from the stomach showing the apparent transformation of the outer zone of the mucigenous border of Fig. 17 into globules of mucous (a). (b) Mucigenous border. (c) Nucleus. Hardened by Erlicki's fluid. Stained with hematoxylin and eosin. (× about 300).

FIG. 19. Surface view of the epithelium cells of the stomach, showing the hexagonal outline of the ends of the cells. Isolation in $\frac{1}{10}$ osmic acid. (× about 165).

PLATE IV.
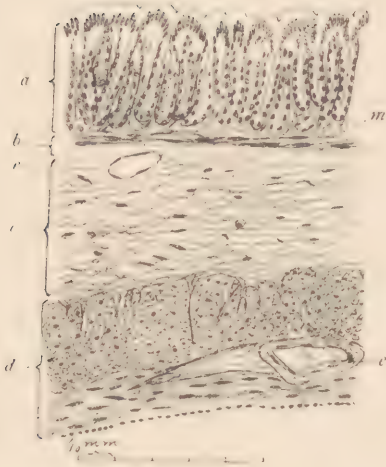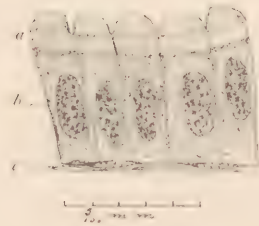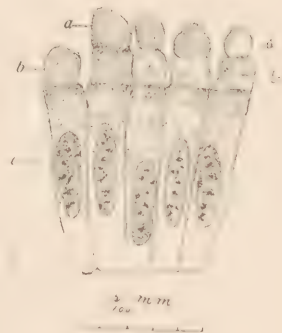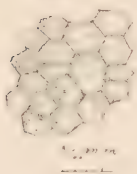
PLATE V.

FIG. 20. (× about 300). Two gastric tubules from the middle region of the stomach. (a) Surface cells of the stomach with their mucigenous border (b). (c) Neck of the gland. (d) Mucous cells between the neck and the fundus of the gland. (e) Secreting cells of the fundus of the gland. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 21. (× about 165). A gland from the esophagus just cephalad of the stomach, showing the large number of mucous cells, and three secreting tubules. (a) Surface epithelium. (b) Neck of the gland. (c) Mucous cells. (d, d, d) Tubules of secreting cells. Hardened by mercuric chlorid. Ehrlich-Biondi stain.

FIG. 22. (× about 300). A transection through the fundus of a gastric gland, showing the ental granular zone (a). Hardened in Erlicki's fluid. Ehrlich-Biondi stain.

PLATE V.

20

21

22
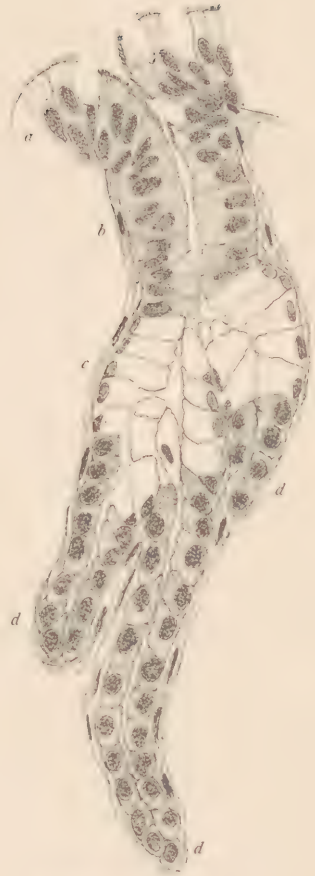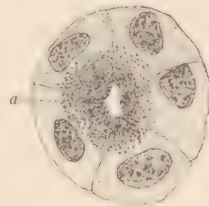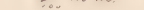
PLATE VI.

FIG. 23. (× about 280). Surface cell from the epithelium of the stomach, showing fat granules (a). (b) Mucigenous border. (c) Nucleus. Isolation in $\frac{1}{10}$ osmic acid.

FIG. 24. (× about 280). A longisectiou through a pyloric gland. (a) Surface cells. (b) Mucigenous border. (c) Gland cells. The outlines of the cells were indistinguishable in the gland drawn, and were put in from isolated specimens. (m) Connective tissue. Hardened in picric alcohol. Stained with hematoxylin and eosin.

FIG. 25. Isolated cells of a pyloric gland. These cells were found in the position here shown. Attention is called to the long processes extending between adjacent cells. Isolation in $\frac{1}{10}$ osmic acid.

FIG. 26. (× about 280). A vacuole containing a large leucocyte from the epithelium of the stomach. (a) Mucigenous border. (b) Large leucocyte. (c) Small leucocyte. (m) Subepithelial tissue. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 27. (× about 280). A gland from the intestine near the opening of the bile ducts. (a) Surface cells with striated free border. (g) Goblet cell. (b) Gland. (m) Connective tissue. Attention is called to the lumen of the gland. The outlines of the cells were not distinct in the section, and were put in from isolated specimens. Hardened in Erlicki's fluid. Stained with hematoxylin and eosin.
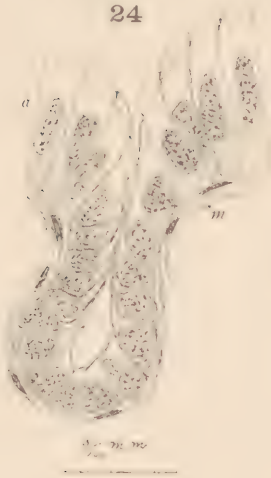
FIG. 28. (× aboat 280). Cells of the intestinal gland. Isolation in equal parts of water and Müller's fluid.

FIG. 29. (× about 300). Surface view of the intestinal epithelium. (g) Constricted top of the goblet cells. The deeper outline of the goblet cells is indicated by dotted lines. (a) Polygonal ends of the columnar cells. Hardened in mercuric chlorid. Ehrlich-Biondi stain.
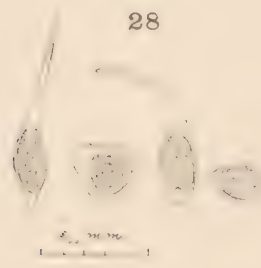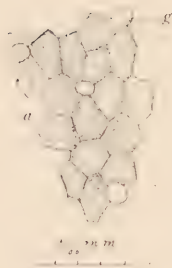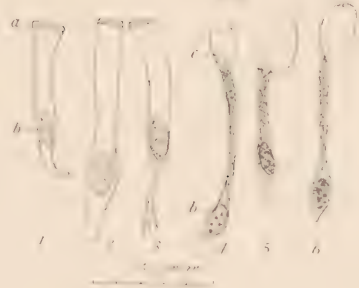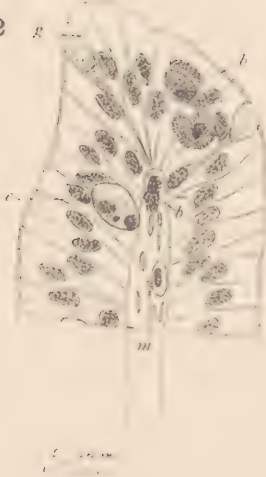
PLATE VI.

PLATE VII.

FIG. 30. (× about 30). Transverse vertical section through the wall of the intestine, showing the transected longitudinal folds, the scattered intestinal glands, and the general relations of the coats. (a) Epithelium of the surface. (b) Glands. (m) Submucosa. (d) Circular muscular coat. (e) Longitudinal muscular coat. Hardened in mercuric chlorid. Stained with hydrochloric acid carmine.

FIG. 31. (× 75). Transverse section through two folds of the intestine, showing the surface epithelium and two glands (b, b), situated one between the folds, the other upon the lateral side of one of the folds. (a) Epithelium of the surface. (g) Goblet cell. (m) Connective tissue. Hardened in mercuric chlorid. Hydrochloric acid carmine stain.

FIG. 32. (× about 165). A transection of the crest of a fold of the mucosa (g) Goblet cell. (b, b, b) Yellow granuled leucocyte. (m) Connective tissue of the fold. Hardened in mercuric chlorid. Hydrochloric acid carmine stain.

FIG. 33. (× about 165). Epithelium of the intestine from the lateral surface of a fold, containing leucocytes with enclosed red globules. (a) Leucocytes. (b) Red and green globules (c) Striated border. (g) Goblet cells. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 34. (× about 300). Isolated cells of the intestinal epithelium. 1, 2, 3. Columnar cells. 4, 5, 6. Goblet cells. (a) Striated border. (b) Nucleus. (c) Theca of the cell. From dissociation in equal parts of water and Müller's fluid.
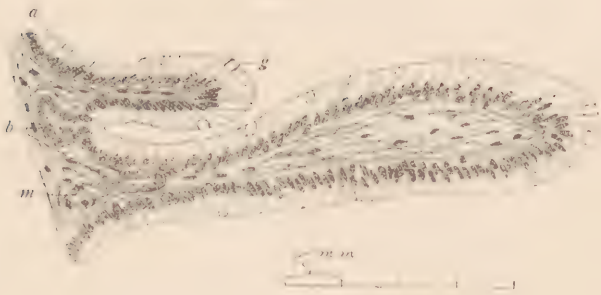
PLATE VII.

PLATE VIII.

FIG. 35. (× about 280). Leucocytes found in the epithelium and subepi-thelial tissue throughout the enteron. (a) Large leucocyte with faintly staining protoplasm. (b, c, d) Large leucocytes containing enclosed glo-bules of matter in various amounts, and also small leucocytes. (e) Large leucocyte from the intestine containing yellow granules, and also a small leucocyte. (x) Cell-body of the small leucocyte. (y) Nucleus. (f, g, h, i) Small eosinophile leucocyte from the submucosa of the stomach. (k, l) Leu-cocytes from the intestine containing yellow granules. (m, n, o, p) Small leucocytes from the intestinal epithelium, showing enclosed globules, simi-lar to free globules (r), found also in the epithelium. Hardened in mercuric chlorid. Ehrlich-Biondi stain.

FIG. 36. (× about 300). Isolated cells from the cloacal epithelium. Mucous cells, and columnar cells. (a) Theca. (b) Nucleus. From disso-ciation in equal parts of water and Müller's fluid.

FIG. 37. A transverse vertical section through the dorsal wall of the cloaca of a female Necturus to show glands containing zoosperms. (a) Surface epithelium of the cloaca. (b) Tubular glands lined with columnar epithe-lium. (c, c, c, c) Flask-shaped glands containing zoosperms. (d) Transect-ed tubular glands (b) (m) Connective tissue. Hardened in picric alcohol. Stained with hematoxylin and picric alcohol.

FIG. 38. A diagram showing the relations of the bile and posterior pancre-atic ducts, constructed from serial surface sections of intestine and adjacent parts of the pancreas and liver. (p.d., p.d.) Pancreatic ducts. (h, h, h, h, h) Hepatic ducts. (c, d) Cystic duct.)g) Gall-bladder. (a) Single ana-stomosis of the cystic duct with the hepatic ducts. Hardened in mercuric chlorid. Hydrochloric acid carmine stain.

PLATE VIII.

35

a b c d e

f g h i k l

m n o p r

37

36

38

Liver

G c d h

Pancreas

a

p d p d

Intestine